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For:

PARMACEUTICAL COMPOSITIONS COMPRISING NATURAL HUMAN ALPHA-INTERFEON

DECLARATION UNDER RULE 132

I, *Renzo Brozzo*

DECLARE

that I was born in Perugia 12.21.1947, of Italian nationality, graduated in Biological Sciences – Phd in microbiology.

(CURRICULUM VITAE)

I took a degree in Biological Sciences at the University of Perugia, from 1972.

Author of publications and patents in the field of Biological Derivative.

From 1972 till 1974 served in the army.

In 1974 I was a Researcher and then Head of the Research Laboratory at the PROTER S.p.A. Company for the production of Anti-viral Biological Derivatives up until 1980.

From 1980 till 1986 I have been working at VIROGEM AG in Basel, Switzerland as Director of Production I am author and co-author of thirty patents all concerning the Antibiotics and antiviral Production for human use.
During the permanence in Switzerland I've also published, among many, the "Evaluation of Human Fibroblast Interferon in Patients with Metastatic Breast Cancer" published by The American Society for Microbiology (1982), Acts of the XII congress of Current Chemotherapy and Immunotherapy (see copies herewith attached). From 1990 till now I am Research and Development Manager at the Istituto Farmacoterapico Italiano S.p.A.
In this capacity, and as a co-inventor of the application as per re, I carried out tests to evaluate the safety and effectiveness of peroral interferon in its liquid form with respect to the solid form in the treatment of adult subjects (18 years or more) affected with chronic hepatitis non-A and non-B or C with compensated hepatic disease.

30 patients were randomised and assigned in three groups equally in the following manner:

Group I – 10 subjects treated with interferon in liquid form (vial)
Group II – 10 subjects treated with interferon in solid form (tablet)
Group III – 10 subjects treated with placebo (This group was divided in two subgroups, 5 subjects treated with liquid form while the remaining 5 subjects were treated with the solid form).

The normalisation of serum alanine aminotransferase levels (ALT) was taken into consideration as the primary efficacy parameter (historical valuation), during and at the end of the therapeutic cycle.

Monitored Parameters

A) <u>Biochemical Evaluation of the therapeutic response (ALT Analysis)</u>

The criteria for the evaluation of the therapeutic response and any eventual relapse was established in the following manner:

- The ALT serum levels were taken into consideration as stable indicator of hepathic cell damage;

- The normalisation of serum ALT at the end of the treatment (24 weeks) was defined as a complete response;
- A greater then 50% decrease in serum ALT level that led to less than 1.5 times the upper level of the normal range was defined as the near-complete response;
- A more than 50% decrease of the ALT levels was considered a partial response.

A worsening or relapse is defined as an increase in serum ALT levels grater than 1.5 times superior to the normal following the end of the treatment for complete responders.

For patients with a near-complete response or a partial response, a relapse or worsening is defined as an increase of ALT levels two-fold or a value that is higher or equal to the levels of the patient before therapy.

The worsening or relapse must be confirmed by at least three consecutive ALT determinations, each at least one week apart from the other.

B) <u>Historical Evaluation of the Therapeutic Response (Hepatic biopsy Analysis)</u>

The procedure for the sampling and analysis of the hepatic biopsies are prospectively defined as follows:

- the hepatic biopsies were obtained before and at the completion of the therapeutic cycle (24 weeks);
- in all patients the paired biopsy specimens were reviewed by a single pathologist who was blinded as to the identity of the patient, the group category, and whether the samples being analysed were obtained prior to or after treatment.
- In order to asses the change in hepatic histology, the samples were evaluated using the following procedure:

    ◆ The pathologist does a general assessment of the paired samples, and each pair is evaluated as improved, equal or worse.

It has been noted that the historical state in the periportal and lobular areas can be particularly predictive for the development of subsequent cirrhosis. For this, the changes in this area must be analysed with much attention.

C) Evaluation of safety

Safety was evaluated by questioning and examining each patient for possible intolerance to treatment and adverse systemic effects during the course of the therapy.
The physician judged the relatedness of an adverse experience as probably, possibly, or not related to treatment. Adverse effects were assigned a severity according to the following scale: 1=mild, 2=moderate, 3 =severe, 4=life threatening.

D) Dosage forms

Composition of the peroral liquid form (vial):

Active principle:
Natural human α-interferon 150 I.U./ml/dose

Excipients
Aqueous saline solution
Human albumin as a stabilizer

Composition of solid form (tablet)

Active principle:
Natural human α-interferon 150 I.U./tablet/dose

Excipients:
Human albumin as a stabilizer
Mannitol
Magnesium stearate

Results

Dosage of ALT serum levels

In table 1 the average ALT responses are summarized for the 3 groups (liquid form, solid form, placebo)

The liquid form resulted decisively superior to the solid form and placebo.

With the liquid form, 60% of the patients (6/10) of Group I obtained an ALT reduction of at least 50%, with a median response time of two weeks initial treatment.

Of the six patients that responded to the treatment with the oral form, 4 obtained a reduction of ALT levels to normal, 1 patient to near normal levels, and only 1 achieved a partial response.

All of this compares to an overall ALT response rate of 30%. (3/10) for patients in Group II, treated with interferon in tablet form, and 10% (1/10) for patients in Group III treated with the placebo. The average response time for the responders of Groups I, II and III was approximately 4 weeks.

Historical Analysis of Liver Biopsies

The improvement of the historical frame was evaluated by comparing the pre and post treatment hepatic biopsies through a General Assessment made by the pathologist.

To the patients treated with interferon in the liquid form (vial), a historical improvement was reached compared to the group treated with placebo.

Patients treated with interferon in the solid form (tablet), a minor improvement was achieved slightly superior to the patients treated with placebo.

Moreover, the combined analysis, both the general assessment and the historical state modifications for the three groups in the study demonstrate significant improvement in the patients treated with interferon with respect to patients treated with placebo. The improvement is due primarily to a decrease in the seventy or necrosis and degeneration in the lobular and perportal regions that was observed in 80% (8/10) of the patients treated with interferon in the liquid form (vial), in 60% (6/10) of patients treated with interferon in the tablet form compared to the 20% (2/10) of patients treated with placebo. (Table 2)

Worsening or Relapse

Relapses or worsening of the disease were registered in only 10% (2/20) of the patients treated with interferon, while a worsening was registered in 40% (4/10) of the patients in the group treated with placebo.

Relationship between ALT levels and Historical Improvement

Patients respondent to ALT had a major probability (4 times higher) of having a historical improvement at the biopsy analysis compared to those patients that do not respond positively to ALT.

Prognostic Indicators

ALT responses were seen regardless of the baseline histologic diagnosis.

Adverse Effects

Interferon in its therapeutic form comparing the liquid form vs. the solid form, was well tolerated and non patients reported any serious or life threatening side effects (Table 3). In fact, the most common toxic effects that were foreseen were the non serious flu-like symptoms, fever, headache, myalgia, as most commonly reported in literature, are related to the systemic use of interferon.

Conclusions

Of each of the three groups in the study, the improved ALT response index was observed in Group I with 60% treated with the liquid form of interferon.

Approximately half of the patient responders of the interferon treatment had normal ALT levels for at least 6 months, after the therapy was suspended, indicating that a subgroup of patients (50%) has beneficial effects after an interferon therapeutic cycle (24 weeks).

Significant inprovement was registered in the histological tests in patients treated with interferon with respect to patients treated with placebo.

The improvement is due to a decrease in the severity of necrosis and tissue degeneration of the lobular and perportal regions.

The historical improvement is of 3 or 4 times more frequent in patients with a marked and persistent normalisation of ALT levels.

Lastly, interferon treatment is of 3 or 4 times more frequent in patients with a marked and persistent normalisation of ALT levels.

Lastly, interferon treatment is effective in the treatment of hepatitis and interferon in the liquid form is superior to interferon in the solid form with the same dosage in the normalisation of the ALT level rates, 60% vs. 30% respectively.

Table 1

ALT Response (after 24 week treatment)

Treatment	Patients Treated	Complete Response	Partial Response	No Response	Worsening And/or Relapse	% of Responders
150 I.U/ml/dose Group I liquid form (vial)	10	4	2	3	1	60%
150 I.U/dose Group II solid form (tablet)	10	2	1	6	1	30%
Group III (placebo) (*)	10	1	1	4	4	20%

(*) 5 patients with solid form and 5 patients with liquid form

Table 2

Histological State of Biopsies (pre and Post Treatment)

Treatment	Patients Treated	Complete Response	Partial Response	No Response	Worsening And/or Relapse	% of Responders
150 I.U/ml/dose Group I liquid form (vial)	10	8	1	1	1	80%
150 I.U/dose Group II solid form (tablet)	10	6	3	1	1	60%
Group III (placebo) (*)	10	2	6	6	2	20%

(*) 5 patients with solid form and 5 patients with liquid form

Table 3

Peroral Treatment using nHuIFNα

(Side Effects)

Side Effects		Total n. of cases		Degree of severity	
Increase in appetite	2	1	1		
Decrease in appetite	2	1	1		
Decrease in appetite	1		1		
Asthenia	1		1		
Sense of hunger	1				
Depression	NO				
Weight gain	1	1			
Weight loss	NO				
Dyspepsia	1		1		
Shivers	1		1		
Fever	2		2		
Abdominal pain	2	1	1		
Articular pain	1	1	1		
Muscle aches	2		2		
Alvus regularization	2		2		
Constipation	1		1		
Cystitis	NO				
Sense of disgust towards the drug	3				
Other	NO	NO	NO	NO	NO

Due to the fact that some patients under examination presented more than one side effect, the number of subjects that presented these side effects is notably lower than the cases registered (8/20 patients treated with interferon).

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

04/23/2001
(date)

(signature)

20. A method of treating a subject having viral hepatitis, comprising administering to the subject an oral liquid formulation of natural human α-interferon to be administered through peroral route at a daily dosage between 100 IU and 500 IU.

21. The method of claim 20, wherein the human α-interferon is obtained from lymphoblastoid cell culture.

22. The method of claim 20, wherein the human α-interferon is obtained from lynphocyte cells.

23. The method of claim 20, wherein the formulation is administered in a single dosage unit having a volume of approximately 1 milillliter.

24. The method of claim 21, wherein the formulation is administered in a single dosage unit having a volume of approximately 1 milliliter.

25. The method of claim 21, wherein the formulation is administered in a single dosage unit having a volume of approximately 1 milliliter.

26. An article of manufacture comprising packaging material and a pharmaceutical agent in a liquid formulation within said packaging material, wherein the pharmaceutical agent is therapeutically effective for treating viral hepatitis, and wherein the packaging material comprises a label which indicates that the pharmaceutical agent can be used for treating viral hepatitis and has to be administered through peroral route at a daily dosage between 100 IU and 500 IU, and wherein said pharmaceutical agent is natural human α-interferon.